Florida Citrus Advanced Technology Program

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Genetics	1/19/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Genomic sequencing of Ca. Liberibacter americanus from Brazil and two circular phage genomes of L.asiaticus.

TITLE and CONTACT INFORMATION

Proposal Title	Genomic sequencing to closure of a curated Florida citrus greening strain of Candidatus Liberibacter asiaticus		
Principal Investigator	Dean W. Gabriel	*PI Last Name*	Gabriel
Email	dgabr@ufl.edu	*FDACS Contract Number*	65
Phone	352-392-7239	*Project Duration (years)* 3	*Year of Project* 1
Organization	University of Florida, Gainesville	*Total Direct Funds (current year)*	$246,601.00

REPORT UPDATE (650 words; provide details about your headline)

This project has three objectives: 1) gap closure of Ca. Liberibacter asiaticus (Las) found in Florida; 2) complete genomic sequencing to closure of Ca. L. americanus (Lam) strain São Paulo from Brazil, and 3) comparative genome analysis of Las and Lam to attempt to determine common factors enabling pathogenicity to citrus.

Objective 1 Progress: Within the recently published Las strain psy62 chromosomal genome (Duan et al. 2009), many unique genes of unknown function are found, and several phage related genes are found integrated into the chromosome, but no replicating phage DNA was found. We reported last quarter the finding of two complete circular lytic phage genomes, SC1 and SC2, with the copy number of SC1 replicating an average 10X higher in citrus and 20X higher in periwinkle, than when the phage is integrated into the chromosome in infected psyllids. [Gabriel & Zhang, 2009. Phytopathology 99 (6): S38]. Neither phage were previously reported. Phage SC1 is 40 kb in size. SC2 is 39 kb in size and appears to be a degenerate variant of SC1. Phage particles, 25 nm in size, and likely corresponding with infectious SC1 phage, have now been documented inside Las cells, inside swollen Las cells that appear to be lysing and outside of Las cells by electron microscopy in Las-infected periwinkle phloem cells. Phage particles were not seen in Las-infected citrus. In citrus, the phage are replicating to relatively high copy number but not lysing Las cells, as they are in periwinkle.

Since some of the SC-1 and SC-2 phage genes were found by Duan et al (2009) to be integrated into the Las genome, both phage likely have a lysogenic cycle. Lysogenic phage are known to mobilize bacterial genes when they repackage into infectious particles and then horizontally transfer these genes between different bacteria that they infect. Any genes carried on a phage that replicates to a level 10X higher than the host chromosome also replicates the copy number of all genes it carries to a level 10X higher than the rest, dramatically increasing gene expression levels, possibly affecting virulence.

Following that lead, several new Las genes were found on SC1 and SC2. Detailed genomic analysis has revealed a total of six outer membrane proteins, five of which are predicted to form beta barrel structures, several of which were not previously reported in the psy62 genome. One of these appears to be an enzyme that is predicted to modify the bacterial outer membrane structure; another is a predicted porin, which form structures through which molecules can passively diffuse. A third, and previously unreported protein is a predicted outer membrane anchored protein with a collagen triple helix repeat. This unusual protein may be capable of forming a fibrous protein matrix, perhaps causing Las aggregation and trapping Las within phloem cells. We observed Las cells within periwinkle phloem by EM to occupy well over 50% of the cell cytoplasm.

Objective 2 Progress: In collaboration with Fundecitrus in Brazil, Lam strain "São Paulo" DNA samples extracted from citrus and purified on pulsed-field gels by Dr. Nelson Wulff has resulted in 78 kb of partially confirmed Lam genomic sequence obtained by 454 sequencing assembled into contigs greater than 1 kb. Dr. Wulff recently brought new Lam DNA to our lab for additional sequencing and comparison with the Las genome and the yet to be published sequence from the Liberibacter causing "zebra chip" disease of potato (Objective 3). We are on track to meet target deadlines of the original proposal.